A Review of the Methodology Used to Derive Site Specific Water Quality Criteria for Al, Cu, and Zn in the Chuit River, Alaska

Submitted to the Alaska Department of Environmental Conservation

July 23, 2014

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TOXSERVICES
TOXICOLOGY RISK ASSESSMENT CONSULTING

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Executive Summary

PacRim Coal, LP has petitioned the Alaska Department of Environmental Conservation (ADEC) for site-specific criteria (SSC) for waters within the Chuitna Coal Project area. Tetra Tech conducted toxicity tests used to calculate the USEPA Water-Effect Ratios (WER) for Al, Cu, Pb, and Zn. A Confirmatory test was requested by Region 10 EPA to test the toxicity of a mixture of the four metals at the proposed WER derived SSC criteria (also conducted by Tetra Tech).

ADEC has requested an independent toxicological review of the toxicity test documents produced by Tetra Tech in support of PacRim Coal's petition, including a review of ADEC's draft analysis of Tetra Tech's WER studies, and a review of Region 10 EPA's analysis and comments on Tetra Tech's WER studies. ADEC also requested expert input on responding to two questions: (1) Whether use of the mixed metals Confirmatory tests to derive chronic criteria is appropriate given the chemistry and precipitation problems associated with this test?; and (2) Does the methodology allow the use of individual WER tests to derive site specific criteria, even if the mixed metals Confirmatory tests did not have metals concentrations as high as the individual WER tests?

The current report is authored by Dr. Ruth Sofield, and provides an independent evaluation of the work completed by Tetra Tech, including an assessment of individual metal WER tests and Confirmatory (mixture) tests, and responds to ADEC's questions relating to the use of specific tests to derive SSC. As detailed in the report, Dr. Sofield does not recommend changing the SSC derived from the WERs to SSC derived from the Confirmatory tests. As opposed to using the Confirmatory results to derive SSC, Dr. Sofield recommends that the SSC be derived for Al (as total recoverable) using the single metal WER, and recommends that Cu and Zn criteria be derived (as dissolved) using the individual WERs for those metals.

1.0 Background

PacRim Coal, LP retained Ruth Sofield, PhD through ToxServices LLC, to review documents and provide a technical professional opinion on the site specific water quality criteria (SSC) adjustment for the Chuit River, as part of the Chuitna Coal Project. PacRim has petitioned the Alaska Department of Environmental Conservation (ADEC) for site-specific criteria for waters within the Chuitna Coal Project area. Tetra Tech (Owings Mills, Maryland) conducted the toxicity tests used to calculate the USEPA Water-Effect Ratios (WER) for Al, Cu, Pb, and Zn. A Confirmatory test was requested by Region 10 EPA to test the toxicity of a mixture of the four metals at the proposed WER derived SSC criteria (also conducted by Tetra Tech). Subsequent to the Confirmatory test, the SSC adjustment application for Pb was withdrawn (Tabor, personal communication, May 5, 2014). ADEC has requested that an additional toxicological review of documents produced in support of this petition take place.

1.1 Objectives of this Review

Specifically, the two primary objectives associated with this review as requested by ADEC are:

Objective 1. Expert review of the Water Effects Ratio (WER) studies (original and mixed metal confirmation tests) for the SSC, review of the Alaska Department of Environmental Conservation's (ADEC's) draft analysis of the WER studies, and review of Region 10 EPA's analysis and comments on the WER studies.

Objective 2. Provide a response as a technical professional opinion of the following key questions:

a. Is it appropriate to use the mixed metals Confirmatory tests to derive chronic criteria (as Region 10 EPA suggests) given the chemistry and precipitation problems associated with this test? As described by ADEC "Aluminum makes this approach especially problematic since the original criteria was developed under different pH and hardness conditions and with different species than were used in the mixing metals test. Aluminum also complicates the chemistry for copper in the mixing metals test."
b. Does the methodology allow the use of individual WER tests to derive the site specific criteria, even if the mixed metals Confirmatory tests did not have metals concentrations as high as the individual WER tests?

1.2 Qualifications

Ruth Sofield completed a PhD in Environmental Science and Engineering and a post-doc at the Colorado School of Mines (Golden, CO) with research expertise in aquatic toxicology, and radionuclide chemistry and speciation. Further training included metals based nanoparticle toxicity and chemical speciation. Her expertise is in metal toxicity, with specific emphasis on the effects of environmental conditions on metal speciation and freshwater toxicity. A CV is included as Appendix B.

1.3 Information Reviewed

ADEC requested a third party review of the following documents:

1. USEPA. Use of the Water Quality Effects Ratio in Water Quality Standards. 1994. http://water.epa.gov/scitech/swguidance/standards/upload/2003_08_06_standards_modif-int- wer.pdf
2. Tetra Tech Study Plan for Developing Site-Specific Water Quality Criteria for the Chuitna River Basin, Alaska, Sections 1, 2, 3.2, and 5, April 20, 2009
3. Tetra Tech. Determination of an Aluminum, Copper, Lead, and Zinc Water Effect Ratio for the Chuit River Basin, Alaska. March 12, 2010.
4. Tetra Tech. Memorandum. Additional Discussion of WER Mixture Testing. Aug 10 2012.
5. Unofficial Region 10 EPA comments to the Alaska Department of Environmental Conservation via email from William Beckwith May 10, 2013.
6. ADEC. DRAFT Decision Document. Site Specific Criteria for Bass Creek, Middle Creek, and Lone Creek, Tributaries of the Chuit River. Public Notice Draft. March 29, 2013.

Items two through five were provided by PacRim Coal. An updated draft of item 6 (from 5/13/14) was provided by ADEC. Additional documents were reviewed as cited in this technical professional opinion. Personal communications with Brock Tabor (ADEC), Marcus Bowersox, and Jerry Diamond of Tetra Tech (written comments included as Appendix A) were used to clarify information.

1.4 Structure of this Report

Sections 2.0 through 4.2 are a summary and assessment of the work completed by Tetra Tech. Specifically, Sections 2.0 through 2.2 are a summary and assessment of the individual metal WER tests, Section 3.0 provides background for understanding mixture toxicity, and Sections 4.0 through 4.1 are a summary and assessment of the Confirmatory (mixture) tests. Sections 5.0 through 5.4 are analyses conducted by Sofield to support recommendations, which are included as Section 6.0.

2.0 Summary of the Individual WER Tests Conducted by Tetra Tech

An individual WER was determined for four metals using site water from sampling station 141 on Middle Creek and synthetic water (lab); the synthetic water was very soft for Al and soft water modified to obtain a hardness between 20 and 25 mg/L as $CaCO_3$ for Cu, Pb, and Zn (USEPA 2002, Tetra Tech 2014). The Al lab water was modified to target 10 mg/L hardness and pH 6.5. For Al, the WER is a Total Recoverable cccWER. For Cu, Pb, and Zn, the WER is a Dissolved cmcWER and can be applied to the CCC (Criterion Continuous Concentration) for chronic toxicity. In calculating the WER for Cu, Pb, and Zn, the lab LC50 was hardness corrected to match the hardness of the site water. Three rounds of testing were conducted using site water collected at three flow conditions to represent the range of physicochemical measurements obtained in previous monitoring. *Pimephales promelas* (fathead minnows) and *Daphnia magna* were the test organisms; once it was established which of the two was most sensitive, the remaining testing rounds used only that test organism. For Al, *Pimephales promelas* (fathead minnows) were used for the three rounds of testing (discussed below). For Cu, Pb, and Zn, *Daphnia magna* was the most sensitive. For each round, the WER for each metal was calculated with Equation 1 or 2[1].

If LC50 (lab) > SMAV; $WER = \frac{LC_{50}\,(site\ water)}{LC_{50}\,(lab\ water)}$ Equation 1

If LC50 (lab) < SMAV; $WER = \frac{LC_{50}\,(site\ water)}{SMAV}$ Equation 2

where SMAV is the species mean acute value.

The geometric mean of the three WERs is reported as the final WER (Table 1).

2.1 Assessment of the Testing Approach and Results

The laboratory toxicity tests for the individual metals were of good design; the appropriate age of organism was used, testing methods followed accepted methods, and QA/QC procedures were good. Several irregularities were noted.

1. The measured dissolved Zn concentrations (for all sampling rounds) and the measured dissolved Al (for the third sampling round) were greater than the measured total metal concentrations in the ambient site waters. This did not impact final toxicity results or interpretation of those results since the concentrations of the spiked samples, not ambient samples, were used for the statistical analysis of the LC50s.
2. The *D. magna* control (lab water) survival was lower (60% and 80%) than test acceptability criteria allows for ($\geq$90%, USEPA 2002) in the first and second rounds of Al testing. Additionally, there was no correlation between the measured Al concentrations and the

[1] Equation 1 was used for Al.

percent mortality for the second round of tests in the lab water and so the concentration response curve is not as expected, i.e. effects do not increase as Al concentration increases (Tetra Tech 2010, Table 3.3a). Tetra Tech proposed that the low hardness (10 mg/L) in the control is the cause of the unacceptable control mortality and lack of a positive correlation, which is a reasonable explanation. The concentration-response curve is also not as expected for the round 2 site water with *D. magna* (98 mg/L had greater survival than the five Al concentrations < 98 mg/L and the 2 concentrations > 98 mg/L, see Tetra Tech 2010, Table 3.3b).

No WER was calculated from these two *D. magna* tests; using the methodology for determining the final WER detailed in Tetra Tech[2] (2010) this would only impact final WER calculations if *D. magna* was more sensitive than *P. promelas*. It is not clear from the results of these tests which is the most sensitive species under these conditions.

[2] The final WER was calculated as the geometric mean of three WERs from the most sensitive species tested. USEPA (1994) requires that three WERs be determined for a primary species and only one confirmation WER be determined for the secondary species.

Table 1: Summary of criteria including unmodified criteria and proposed site specific criteria compared to metals concentrations used in the Confirmatory test

	From single metal WER	Current AK Standard (µg/L)[b,d]			Tetra Tech Recommended SSC (µg/L)					ADEC Recommended SSC (µg/L)			Region 10 EPA Recommended SSC (µg/L)			Confirmatory Concentrations in 80% Spike of Site Water[m] (µg/L)				
	final WER[a,b]	CMC	CCC	Hardness	CMC[b,c]	CMC[e]	CMC[f]	CCC[b,c]	Hardness	CMC[b,h]	CCC[b,h]	Hardness	CMC[i]	CCC[j]	Hardness	*D. magna* (total)	*P. promelas* (total)	*D. magna* (dissolved)	*P. promelas* (dissolved)	Hardness
Al	7.48	750	87	25	750	750	651	651	NA	750	651	NA	<681	NA	NA	700	662.5	122.8	118	unknown
Cu	6.17	3.64	2.74	25	22.46	23.4	37.7	16.9	25	22.46	16.9	25	<15.2	<20.19	unknown	35.5	37.8	14.5	15.8	unknown
Pb	8.88	13.88	0.54	25	123.25	124	211.5	4.8[g]	25	NA[k]	NA[k]	NA	NA[k]	NA[k]	NA	322.5	337.5	23.5	30.3	unknown
Zn	1.17	36.2	36.5	25	42.36	43.3	61.8	42.7	25	42.36	42.7	25	<32.8	<33.07	unknown	65.3	67.8	30.8	34.8	unknown

[a] Based on *Daphnia magna* for Cu, Zn, and Pb. Based on *Pimephales promelas* for Al. Calculated as the geometric mean of three acute toxicity tests. From Tetra Tech (2010).

[b] Total metal concentration used for Al and dissolved metal concentration for Cu, Pb, and Zn.

[c] From Tetra Tech 2010. Calculated as the (Final WER) * (Current AK Standard)

[d] From ADEC 2008.

[e] As total metal concentrations. From Diamond and Latimer (2010a). Calculated as (Tetra Tech recommended CMC (dissolved)) * (ADEC CF)).

[f] As total metal concentrations. From Diamond and Latimer (2011). Hardness is 38 mg/L (Tetra Tech 2014).

[g] Corrected value based on (final WER)*(CCC)=4.795, given as 4.75 in Tetra Tech 2010.

[h] From Table 1, ADEC 2014

[i] Measured as the mean of concentrations from the spiked *D. magna* and *P. promelas* Confirmatory tests. As total recoverable for Al and dissolved for Cu and Zn. From Beckwith (2013).

[j] Uses the same relationship that exists between the CMC and CC of the Alaska standards. Both as dissolved concentrations. From Beckwith (2013).

[k] Request for SSC for Pb rescinded by PacRim Coal.

[m] From Diamond and Latimer 2011.

NA - Not Applicable

3. For all metals, the concentration series was changed from round to round to account for toxicity tests of previous rounds. This is good practice since the goal is to use test concentrations that bracket the effect level of interest (i.e. 50% mortality) and ideally contains partial effects (percent mortality other than 100% or 0% in test concentrations).
4. The reporting limits for water chemistry are reported in Table 13 of PacRim Coal and Tetra Tech (2009). A determination of the method detection limits and practical quantification limits for each analysis is preferable; if this information was reported, it is not included in the reviewed documents. Using the reporting limits, there are two occasions when Cu is below the limits (round 2 and 3 for *D. magna* lab water); this should not impact the LC50s since these are the lowest concentrations used to model the concentration response curve and higher concentrations have minimal mortality so the statistical model will be negligibly affected by the exact value used for these two concentrations.

The approach for Al testing and application of the WER was different from Cu, Pb, and Zn. Specifically, the ambient pH and hardness were adjusted in the lab water (as discussed in Section 2.0). The supporting argument given by Diamond and Latimer (2010a) for this adjustment is that the chronic *Ambient Water Quality Criteria for Aluminum* was based on two toxicity tests conducted in waters with pH approximately equal to 6.5 and hardness of approximately 10 mg/L (USEPA 1988). The result of this is that the SMAV could not be used to determine the WER since the physicochemical conditions under which the criteria were calculated are different than the waters used for the Al toxicity tests. Because the intent of the WER is to determine if site waters modify toxicity when compared to the waters used to derive the criteria (i.e., lab or synthetic water), it is appropriate to use the (low pH and hardness) adjusted lab water to determine the lab LC50 in the WER calculation for the chronic criterion adjustment; this was a valid approach to determining this WER.

The acute criteria from the Al derivation document (USEPA 1988) uses a wider range of pH and hardness conditions with the pH range from 7.05 – 8.3 and hardness at 220 mg/L for the *D. magna* LC50 (and SMAV) of 38.2 mg/L and the pH range from 7.2 – 8.15 with hardness at 220 mg/L for the *P. promelas* LC50 (and SMAV) at 35 mg/L; because of these differences in the waters used to derive the acute and chronic Water Quality Criteria (WQC) for Al, the use of the WER calculated with lab water adjusted to match the chronic derivation of the criteria (i.e., hardness of 10 mg/L and pH 6.5) to derive an acute criterion is not appropriate.

The one issue of concern with any of the Individual tests is the lack of toxicity results from the lab water *D. magna* test for Al since the approach used by Tetra Tech was to use the most sensitive species to determine the WER and it is not clear which is more sensitive under these test conditions. An analysis of considerations is presented.

1. Given that the *D. magna* used in these two rounds for the Al tests were the same as the ones used for the Cu, Pb, and Zn tests – and the control survival was acceptable for those 9 tests (3 metals, 3 rounds), the low control survival is isolated to the Al tests.
    a. The hardness of these tests at 10 mg/L was lower than recommended by USEPA (2002) for culturing (160-180 mg/L) or for testing (80-100 mg/L) and Daphnids are known to be sensitive to hardness (Sofield and Burtini, in press).
2. The lack of a correlation between percent mortality and Al concentration in the round 1 and 2 lab water *D. magna* studies is likely a result of complicated Al speciation and limited Al solubility that is greatly affected by pH. Because of this and the previous consideration, it is my opinion that repeating these tests under these conditions (pH and hardness) would not produce acceptable results according to USEPA (2002) acceptability criteria.
3. From the derivation document for the WQC for Al (USEPA 1988), a single study by Kimball (1978) reported an LC50 and EC50 for the two test organisms used by Tetra Tech. Comparing toxicities from one study is the best approach for understanding relative species sensitivity of these two organisms since similar conditions were presumably used for the tests, although the pH was different in each acute test (7.05 versus 7.34 for *D. magna* and *P. promelas*, respectively), which will affect the toxicity results (Sparling et al. 1997).
    a. The LC50 (and SMAV) was 38.2 mg/L and 35 mg/L for *D. magna* and *P. promelas*, respectively making *P. promelas* more sensitive.
    b. EC50s, however, showed that *D. magna* was more sensitive during chronic exposures (0.7422 and 3.288 mg/L for *D. magna* and *P. promelas*, respectively). Again, the pH of these two chronic tests were different with the *Daphnia* pH = 8.3 and *P. promelas* pH = 7.24 – 8.15. Aluminum solubility (and resultant toxicity) should increase above pH 8 and below pH 6 (Sparling et al. 1997) making a comparison of the chronic results highly questionable since solubility was likely lower in the *P. promelas* chronic tests than in the *D. magna* tests.
4. Tetra Tech used the chronic conditions (pH and hardness from the WQC) to represent a worst-case scenario (PacRim Coal and Tetra Tech, 2009); this is a reasonable assertion.

Given these considerations, there are two implementable options. First, the WER for *P. promelas* could be accepted as the WER for the adjustment of the Al Alaska Water Quality Criteria (AWQC). Second, the individual tests for Al could be repeated for both test species using the acute conditions (pH – 7.2 to 8.3 and hardness = 220 mg/L) and the most sensitive species determined for new Al WER calculations. The first option, even without knowledge of which is the most sensitive, is the most conservative option because this is a worst-case scenario (when compared to the acute conditions) in that less modifying factors are present when hardness is low and pH is in the range that increases solubility. Furthermore, repeating the Al tests (option 2) would use different site waters than were used for Cu, Pb, and Zn, which could further complicate the interpretation of results. Finally, since the CMC (Criterion Maximum Concentration) for Al is not being modified by the WER, using hardness and pH from the acute WQC would be of questionable application to the CCC since the hardness and pH are different in the chronic WQC. It is my opinion that the geometric mean of the *P. promelas* tests should be accepted as the final WER for Al.

2.2 Conclusions on the Use of the WERs

In accordance with the Interim Guidance (USEPA 1994) the cmcWER derived for Cu, Pd, and Zn by Tetra Tech can be applied to both acute and chronic criteria. They should be applied to dissolved criteria because:

1. The Cu, Pb, and Zn WERs were determined for dissolved metals,
2. The toxic species of these metals is established to be in the dissolved fraction based on current scientific understanding of the mechanism of toxicity, and
3. The dissolved to total ratios varied between site water tests and from the USEPA correction factors so that no single correction factor will allow for an accurate conversion between total and dissolved metals (Table 2).

The Al WER should be applied to the total recoverable criterion for the chronic criteria because:

1. The Al WER was determined for total metals using pH and hardness conditions similar to the toxicity tests used to derive the USEPA (1988) and ADEC (2008) CCC,
2. The conditions used to derive the CMC for Al are different than those used to determine the WER and the differences include pH which has a large impact on toxicity in the ranges used for all of the toxicity tests for Al (USEPA 1988, Tetra Tech 2010, Diamond and Latimer 2011),
3. At pH 6-8, the solubility of Al is low (see Section 5.2.1) and so the majority of the Al should be as total recoverable and specifically would not be dissolved, and
4. The USEPA (1988) and ADEC (2008) CCC uses total recoverable Al.

Table 2: Summary of dissolved to total ratios for Cu, Pb, and Zn taken from USEPA (2009) criteria

Metal	Dissolved:Total Ratio			
	From USEPA Criteria	From single-metal WER testing in lab water	From single-metal WER testing in Chuitna water	From Mixture testing
Cu	0.96	0.90	0.81	0.42
Pb	0.93	1.02	0.49	0.08
Zn	0.98	0.98	0.77	0.50

Table from Diamond and Latimer (2011, Table 1; hardness used for Pb USEPA Criteria is 38 mg/L). ADEC (2008) uses the same Conversion Factor for the Dissolved to Total ratio as USEPA.

3.0 Mixed Metals Background

Using the results from the individual toxicity tests for Al, Cu, Pb, and Zn, Region 10 EPA requested a mixed metals Confirmatory test. The Interim Guidance for determining metals WERs (USEPA 1994) includes a special section for dealing with multiple metal situations and is where the Confirmatory test is described. According to the USEPA, the issues associated with mixtures include additivity and synergism[3] of the metals at the site of toxic action, and synergism that results from the metals competing for the same complexing ligands which reduces the total concentration of ligand binding sites and increases the total bioavailable metals concentrations. Importantly, not discussed by the USEPA is the potential for antagonism where the observed toxicity of a mixture is lower than would have been predicted based on individual toxicities. Antagonism can be categorized into functional antagonism, chemical antagonism, dispositional antagonism, and receptor antagonism (Newman 2009). Of particular relevance here is chemical antagonism where two toxicants react with each other to produce a less toxic product; for example, this can occur when chemical reactions cause precipitation of metals (see Sections 5.2.1 and 5.3). The importance of considering these joint actions is well established since criteria based on a single toxicant may be considered protective, but waters that contain multiple toxicants at “safe” levels individually may each contribute enough so the criteria are underprotective because of addition or synergism, and similarly through antagonism may be overprotective.

According to the USEPA (1994) the preferred method for considering the issues of potential additivity or synergism of metals is to conduct at least one additional toxicity test using the

[3] Cedergreen (2014) conducted a meta-analysis to identify how frequently synergism occurs with different classes of chemicals. She concluded that “well documented severe synergistic metal-metal interactions” are rare (occurring 3% of the time) despite a large number of studies on metals mixtures. When synergism does occur with metals, it is most often when the metal concentrations are high (mg/L range).

mixture of metals at their proposed new site-specific criteria (i.e., a Confirmatory test). Acceptability of the mixture test must be demonstrated. As discussed by Diamond and Latimer (2010a) this Confirmatory test was conducted with *D. magna* and *P. promelas*. Acceptability was tested using an ANOVA to calculate the pMSD (13.4%) and a non-parametric Equal Variance t Two Sample Test to compare the mortality in a site sample spiked with the four metals and diluted to 80% with an unmodified (ambient) site sample (CETIS 2011, Tetra Tech 2014). An unspiked lab water sample was used as an additional control.

4.0 Summary of the Confirmatory Tests conducted by Tetra Tech

There were several deviations in the Confirmatory test design from the individual WER test (Tetra Tech 2010, Tetra Tech 2014).

1. The ambient pH and hardness were used for these tests despite addition of Al to the mixtures; Al was tested with modified conditions in the individual WER tests. A comparison of the Al concentrations in the Confirmatory test to the Al CMC would be appropriate because the water conditions are similar.
2. The number of *Daphnia* tested per water type was increased to 40, compared to 20 in the individual WER tests.
3. Only one sample of each water type (lab water, site water, and spiked site water) was tested using *D. magna* and *P. promelas*.
4. The spiked site water had Al, Cu, Pb, and Zn added at target concentrations equal to the (total) site specific criteria proposed based on the individual WERs (Table 1).
5. The toxicity test for the spiked sample used an 80% dilution (20% ambient site water with 80% of the spiked sample). Total recoverable and dissolved metals were measured in the 80% dilution (Appendix A).

A ratio of the measured metal concentrations of dissolved and total fractions were presented by Tetra Tech for Cu, Pb, and Zn (reproduced as Table 2); these ratios are presented for the individual WERs in lab and site water, and in spiked site water for the Confirmatory test. They are the average ratios of all test concentrations and all rounds for either lab or site water and for each metal (Tetra Tech 2014). There is a decrease in this ratio, indicating that a larger percentage of the metals are total, for all individually tested metals when the site water (Chuitna) is compared to lab water. There is also a decrease in this ratio for each metal (Cu, Pb, and Zn) when the individual test site water is compared to the mixture test site water (possible reasons for this are discussed in *Section 5.2*).

For the *D. magna* test, the site water contained ambient concentrations of total Al and dissolved Zn that were 31 and 11.3%, respectively, of the proposed site specific criteria (SSC).

In the spiked site water, the total recoverable Al, Cu, and Zn were within 10% of the proposed SSC (total). The total recoverable Pb concentration exceeded the proposed (total) SSC by 52.4%. The dissolved Cu, Pb, and Zn were 40.1, 11.9, and 50.9% of the proposed (dissolved) SSC (Table 2 in Diamond and Latimer 2011). Similar results were obtained in the *P. promelas* test (Table 3 in Diamond and Latimer 2011). The percentages of *D. magna* and *P. promelas* that survived the exposures are included as Table 3.

Table 3: Percent survival from a 48 hour exposure

	D. magna			*P. promelas*		
	Lab Water	Site Water	80% Spiked Water	Lab Water	Site Water	80% Spiked Water
Replicate 1	100	100	60	100	100	100
Replicate 2	80	100	90	100	100	100
Replicate 3	100	100	90	90	100	90
Replicate 4	100	100	100	100	100	100
Average	**95**	**100**	**85**	**97.5**	**100**	**97.5**

Percent survival from a 48 hour exposure in soft synthetic lab water, ambient site water, and an 80% dilution of site water spiked with Al, Cu, and Zn concentration within 10% of the proposed site specific criteria (SSC) for *D. magna* and 12% for *P. promelas* as total recoverable metals. Pb was at 152.4 and 159.5% of the SSC as total recoverable metals for *D. magna* and *P. promelas*, respectively (CETIS 2011, Diamond and Latimer 2011).

4.1 Assessment of the Testing Approach and Results

The report for the Confirmatory test was abbreviated (Diamond and Latimer 2011) and so clarifications were made by Tetra Tech (2014, included as Appendix B). Based on the report and the clarifications, I am satisfied that the Confirmatory tests were of good design.

The unofficial Region 10 EPA comments (Beckwith 2013) question the analysis used to determine statistical differences between the site water and the spiked site water. The toxicity results for the site waters for *D. magna* and *P. promelas* have high precision (all replicates had 100% survival for both species); statistical tests based on analyzing means relative to the variability of the measurements (such as a t-test) cannot be conducted with this kind of result. An ANOVA, which can be used for three or more samples, followed by pairwise comparisons can be conducted with this data although assumptions of homoscedasticity and a Gaussian distribution are likely violated (n is too small to confirm) and this is not recommended for mortality data (Environment Canada 2007). Beckwith (2013) recommends the Test of Significant Toxicity as a possible statistical test. Other statistical tests for single concentration tests with mortality as the endpoint are included in Environment Canada (2007). In earlier documents, Tetra Tech planned to use a t-test to analyze the data (i.e., Diamond and Latimer 2010a), but the lack of variability in the site control led to the ANOVA and equal variance t test two sample test. It may be useful to consult a statistician to confirm analysis of the Confirmatory test results is acceptable if this continues to be an issue of concern.

The parties involved in this SSC adjustment do not agree how to use the results of the Confirmatory tests. It is my opinion that the important scientific issues center on: 1) the use of total or dissolved metals for the Confirmatory tests and for the final SSC; and 2) mixture chemistry and toxicity. These are discussed in the remainder of this Section and in Section 5.

Diamond and Latimer (2010a) proposed that the proposed SSC as total metal concentrations be used in the Confirmatory tests instead of the dissolved metal concentrations used in the Cu, Pb, and Zn WERs, which was accepted by Region 10 EPA. Diamond and Latimer supported this with several arguments:

1. The site specific acute and chronic Cu, Pb, and Zn criteria calculated using the single metal WERs were similar regardless of whether they were reported as total or dissolved metal concentrations. For confirmation on these calculations, see Question 4 in Appendix A (Tetra Tech 2014).
2. The ADEC (2008) total to dissolved acute and chronic conversion factors for Cu, Pb, and Zn are all near one (0.96 and 0.978 for acute Cu and Zn, respectively; Pb is hardness dependent, at 38 mg/L for example, it is 0.932) . An Al conversion factor is not listed. See Question 2 in Appendix A (Tetra Tech 2014).
3. It is not feasible to obtain the correct dissolved metals concentrations in the mixture.

The first two arguments, which seem to be the same (Appendix A), are used to support that similar results would be obtained for Cu, Pb, and Zn regardless of whether total or dissolved concentrations are used. The third argument was supported (empirically) with the results of the Confirmatory test, where total metal concentrations did not achieve predictable dissolved metal concentrations (Table 2). To add conservatism to the SSC, Tetra Tech further recommends that site specific water quality for Cu, Pb, and Zn be based on the total recoverable metals concentrations (Diamond and Latimer 2010a). Specifically, they recommend that AWQC for dissolved metals be converted to total recoverable concentrations and the appropriate WER applied to those AWQC. Concentrations in the field would then be measured as total metals and used to determine compliance with the AWQC. See Questions 2 and 3 in Appendix A (Tetra Tech 2014).

5.0 Additional Information and Analysis

In support of this evaluation, a brief review of the literature related to mixture toxicity, a summary of aqueous metal chemistry and toxicity, and calculation of toxic units were conducted by Sofield.

5.1 Joint Action of Metals Toxicity

A brief discussion of empirical results of mixed metals toxicity is presented to support a limited understanding of the effects of mixed metals or joint action within the scientific community, particularly as it relates to predictions of effects. Generally, non-interactive metals with similar modes of action are considered to be additive. Copper, Pb, and Zn all interact with a Biotic Ligand (Cu interacts with the Na^+ ion uptake channels, and Pb and Zn interact with the Ca^{2+} ion uptake channels, Niyogi and Wood 2004) but not with each other, so an assumption of additivity is reasonable. Empirical studies that investigated the four metals used in this Confirmatory test were not found, however, Cooper et al. (2009) used binary and tertiary mixtures of Cu, Pb, and Zn to evaluate the joint action of the metals. In this study, mortality during a 48 hour acute exposure for *Ceriodaphnia dubia* and *Daphnia carinata*, and a 7 day chronic exposure for *Ceriodaphnia* was used to calculate the LC50 and EC50, respectively. Toxic Units of the mixtures were then calculated and used to determine the type of joint action the metals had. For the acute exposures: Pb and Zn were antagonistic for both species, Cu and Pb were synergistic for both species, Cu and Zn were synergistic for *D. carinata* but additive for *Ceriodaphnia*, and all three metals were additive for *D. carinata* but synergistic for *Ceriodaphnia*. For the chronic *Ceriodaphnia* exposures: Pb and Zn were antagonistic, Cu and Pb were also antagonistic, Cu and Zn were additive, and the three metals were antagonistic. As summarized by the authors of this study, other research has found that the type of joint action (additivity, antagonism, or synergism) of metals depends on the toxicity endpoint, the organism species, and the metals concentration in mixture relative to the individual LC50s.

As this supports, it is difficult to predict how metals will behave in a mixture, and more importantly that there may be synergism with one combination (concentration and type) of metals and antagonism with a different combination (concentration and type).

5.2 Overview of the Aqueous Chemistry and Toxicity of Metals

The Biotic Ligand Model (BLM) is a conceptual model of how metals cause acute toxicity. The model provides an important framework for an understanding of metal speciation and resultant toxicity. More detailed explanations of the BLM can be found (e.g. Di Toro et al. 2001, HydroQual 2007). The BLM combines an understanding of metal speciation from aquatic chemistry with an understanding of gill surface chemistry and aquatic organism physiology. In aquatic systems, metals can complex with several types of inorganic (e.g., Cl^-, SO_4^{2-}, OH^-, CO_3^{2-}, and Fl^-) and organic ligands (e.g. fulvic and humic acids which are components of dissolved organic carbon (DOC)). The majority of these metal:ligand complexes are not able to interact with the gill surface and so are not considered the toxic form of the metal. As the concentration of these ligands increases, the concentration of metal:ligand complexes increases and toxicity is reduced. At the gill surface, sites at which the toxic form of the metal (primarily the uncomplexed ionic form) interacts are called the Biotic Ligand. The Biotic Ligand is known to be Na^+ or Ca^{2+} ion uptake channels in the gill membrane (see Section 5.1). Other cations, such as Ca^{2+}, Mg^{2+}, and H^+, can also interact with the Biotic Ligand. Because these cations do not cause toxicity when they are at the Biotic Ligand, an increase in the concentrations of these competing cations decreases the metal toxicity. The toxicity of a water can be predicted using the BLM computer model which speciates the metals assuming equilibrium conditions. The BLM computer model has been or is being developed for Ag, Al, Cd, Cu, Ni, Pb, and Zn (Hydroqual 2014). The USEPA (2007) updated the Ambient Water Quality Criteria for Cu to include the BLM for site specific water quality criteria and is reviewing updates for Zn and Ag using the BLM (Santore et al. 2002, Santore and Paquin 2007). Not considered in the BLM are sorption reactions of metals to suspended particulates, such as clays; sorption of metals to suspended particulates would further decrease the concentration of the bioavailable form of the metals and decrease toxicity.

Based on the decrease in the dissolved to total metal concentration ratios in the site water WERs compared to in the lab water WERs (Table 2), it is clear that site water physicochemical properties resulted in lowered solubility of the metals. The decrease in this ratio for the site water Confirmatory test compared to the site water WER further supports that physicochemical properties of the water result in lowered solubility of individual metals but also that the metal complexations in solution affected concentrations of the other metals. It is not possible to distinguish which of these played a larger role in the decreases in solubility[4] (decreases of Cu, Pb, and Zn were 48%, 84%, and 65%, respectively) because the physicochemical properties of the site water changed from the WER tests to the Confirmatory tests (e.g., the ambient hardness increased from 12, 16, and 18 in the WER tests to 38 mg/L as $CaCO_3$ in the Confirmatory test) and the number of metals added to the site water changed from 1 to 4. However, a combination of the two factors is most likely to have influenced the decrease in solubility.

[4] An assumption here is that the dissolved fraction includes only soluble metals species and that no soluble species are retained by the filter.

5.2.1 Aluminum

Aluminum speciation and toxicity is highly dependent on the pH of the solution with low solubility between pH 6 and 8 (Sparling et al. 1997). At pH near 7, Al concentrations in natural water are low and the free ion form[5] of Al (Al^{3+}) does not exist (Butcher 1988, Thurman 1985). Instead amorphous Al hydroxides are present and precipitate (Thurman 1985); as the waters become more acidic or basic, the solubility of the Al hydroxides increases and so more dissolved Al would be expected. Depending on pH, dissolved Al complexes readily with inorganic ligands such as hydroxides, fluorides and sulfates, and organic ligands such as fulvic and humic acids. These complexes act to keep the Al in solution as colloids or dissolved species but also as biounavailable forms of Al, which reduces toxicity. Calcium in solution has also been shown to reduce toxicity of Al (Butcher 1988).

The presence of aluminum in water can decrease the concentrations of other metals in several ways. Alumina (Al_2O_3) and aluminum hydroxides can act as sorbents of DOC between pH 4 to 9. As these precipitate, the DOC is removed from solution (Thurman 1985). The same concept is utilized in wastewater treatment with the addition of coagulants[6] such as alum ($KAl(SO_4)_2 \cdot 12H_2O$) which readily hydrolyze in water. Both DOC and clays have been shown to enhance these precipitation and flocculation processes. When other metals are bound to the DOC, they are removed with the precipitation or flocculation. Other suspended metal particles not bound to DOC may also be removed by sweep flocculation (Truitt and Weber 1979, Thurman 1985, Zhuang 2008, Fu and Wang 2011). Aluminum oxides may also act as sorbents with other metals sorbing directly to the sorbent, with co-precipitation acting to remove metals such as Cu and Pb from solution (Karthikeyan et al. 1999, Pang et al. 2009).

The mechanism of acute toxicity for Al is proposed as either a chemical mechanism (some of the dissolved species of Al interferes with Na^+ and Cl^- regulation) or a mechanical mechanism (Al hydroxide colloids coagulate on the gill and cause suffocation or irritation). At pH 5 to 6, Al coagulation on gill surfaces is thought to be the cause of toxicity (Nordstrom 1981, Butcher 1988, Peléo 1995, Sparling et al. 1997). Because the presence of Al complicates the predictability of speciation of the other metals and resultant toxicity significantly enough, Gustafsson (2011) recommended that Al^{3+} not be included in the Visual MINTEQ (VMIINTEQ) [7]

[5] Specifically, $Al(H_2O)_6^{3+}$ but often presented as Al^{3+}.
[6] Coagulants can act to neutralize negatively charged particles, which reduces repelling between those colloidal and suspended particles, and allows polymerization and flocculation to occur as large particles are formed. The floc may also collide with suspended particles or colloids and physically drag them down as they precipitate (known as sweep flocculation).
[7] VMINTEQ is a geochemical model used to predict the speciation of substances in water. It is a Windows based version of the EPA's MINTEQA2.

BLM model[8], although Hydroqual is developing an Al BLM (Hydroqual 2014). Gustafsson (2011) further recommends that a BLM model for waters with multiple metals cannot be interpreted with confidence since “the effects of mixed metals on toxicity are rather poorly known.”

5.3 Aqueous Geochemical Modeling of Solutions

Predictions of the metal speciation in a natural[9] water with models such as VMINTEQ are possible when the water has been adequately characterized. The intent of the ambient water quality criteria modifications associated with the proposed Chuit River site-specific criteria was not to predict metal speciation and so the data needed to adequately model these waters are not available. Given knowledge of aqueous chemistry, however, general predictions can be made as to the types of interactions that may be occurring in the site waters which may affect the concentrations of the dissolved fractions of the metals and subsequent toxicity to aquatic life. The following discussion is not comprehensive (for example the complex interactions of pH on aquatic chemistry and metal complexation at the Biotic Ligand are not included), but does begin to describe the difficulties in predicting the chemical mechanisms of metal solubility and toxicity in these waters.

Competition for binding sites on DOC occurs when competing cation concentrations such as Ca^{2+}, Mg^{+}, and H^{+} increase, but also when ionic forms of other metals are present. Using the Stockholm Humic Model (SHM) in VMINTEQ (version 3.0) at pH 7, preferential binding of the metals to DOC[10] is Cu> Pb > Al > Zn. As DOC increases in waters, more metals would be expected to be bound to the DOC; as more metals and other competing cations are added, however, a smaller percentage of each individual metal would be bound to the DOC. Similar types of competition between the metals for inorganic ligands would be expected. Coagulation and flocculation would decrease metals concentrations in solution as discussed in Section 5.2.1, although the presence of floc was not reported in any of the reports reviewed in the WER or Confirmatory tests. Precipitation of insoluble non-crystalline minerals is expected at neutral pH, particularly for Al and possibly Pb. As these metals precipitate, there is less competition for complexation with inorganic ligands or binding sites on DOC because that precipitated metal is removed from solution, but the DOC may precipitate also resulting in less overall metals in

[8] Several versions of the BLM exist. The USEPA has accepted a version that uses only Cu. VMINTEQ uses the same databases and calculations, but also includes the ability to speciate many metals in solution, which the USEPA version does not.
[9] “Natural waters” is a term commonly used in aquatic geochemistry to describe waters as they exist in the environment. In this case, the site waters used in the WER and Confirmatory tests are examples of natural waters. See Thurman (1985).
[10] For simplicity, a distinction is not made throughout this document between dissolved organic matter (DOM) and DOC (see Thurman 1985 and Gustafsson 2011 for more discussion on DOM).

solution. As these examples suggests, all of these reactions (and many more) can occur and which one does depends on what else is present in the water.

To provide a more comprehensive demonstration of the complexities, VMINTEQ (version 3.0) was used to speciate a theoretical solution. This solution is based on the Confirmatory test in that it has the same DOC, hardness, alkalinity and ammonia concentrations, but uses the USEPA (2002) recipe for soft water to include K, Cl, SO_4, and Mg. As part of the speciation in the model, precipitation was allowed for oversaturated solids each time a mineral precipitated or dissolved (Gustafsson 2011). Temperature was held constant at 24^{o}C (the temperature used in the Tetra Tech toxicity tests). In all cases, pH was 7.0[11]. The SHM in VMINTEQ was used for the DOC complexations with default parameters[12]. The model was used to calculate carbonate species from the measured alkalinity. Metal concentrations were included as the total metal concentrations added to the site water in the *Daphnia magna* Confirmatory test. The model was run with A) all four metals; B) Al, Cu, and Zn; C) Cu, Pb, and Zn; D) Cu only; and E) Zn only.

When all four metals were included in the model, 99.989% of the Al precipitated as diaspore; 99.981% precipitated when no Pb was present. Precipitation did not occur in the other models. Of all of the dissolved species for each metal (which includes colloids but not precipitated species), the percent that was bound to DOC is included as Figure 1. Zinc and Al were most impacted by the presence of other metals (fewer metals resulted in more of the Zn or Al bound to DOC); this is explained by the preferential binding of Pb and Cu to DOC which out-compete the Zn and Al for binding sites when they are present.

The concentrations of the free ion (toxic) forms of Cu[13], Pb, and Zn were also calculated with the VMINTEQ models described above (Figures 2 and 3). Lower concentrations of the Cu^{2+} and $CuOH^{+}$ were calculated when Pb was absent, which can be explained by more available binding sites on the DOC for Cu when Pb is absent. These two forms of Cu were predicted to be at their lowest concentrations when only Cu was present in the water, for example, the Cu^{2+} (as a percent of dissolved species) was 0.166 when Cu was the only metal, 0.217 when no Al was present, 0.209 when no Pb was present, and 0.217 when all metals were present. Again, this can be explained by less competition for organic and inorganic ligands; more Cu is bound to ligands instead of dissolved as the toxic form when less competing metals are present. Of note, the presence of Al did not change Cu complexation (comparing models A and C). This can be explained by the fact that nearly 100% of the Al precipitated meaning there was negligible

[11] The stated pH of Chuit waters in Diamond and Latimer (2011).
[12] Specifically, 82.5% of the DOC is fulvic acids, the DOM:DOC ratio is 1.65, and 100% of the active DOM is DOC (Gustaffson 2011).
[13] See de Schamphelaere and Janssen (2002).

competition for binding sites between Al and Cu. Similarly, Zn^{2+} concentrations were lowest when Zn was the only metal in solution and Al did not change Zn^{2+} complexation.

Figure 1: Percent of each metal species that was dissolved and bound to DOC out of all dissolved species for that metal.

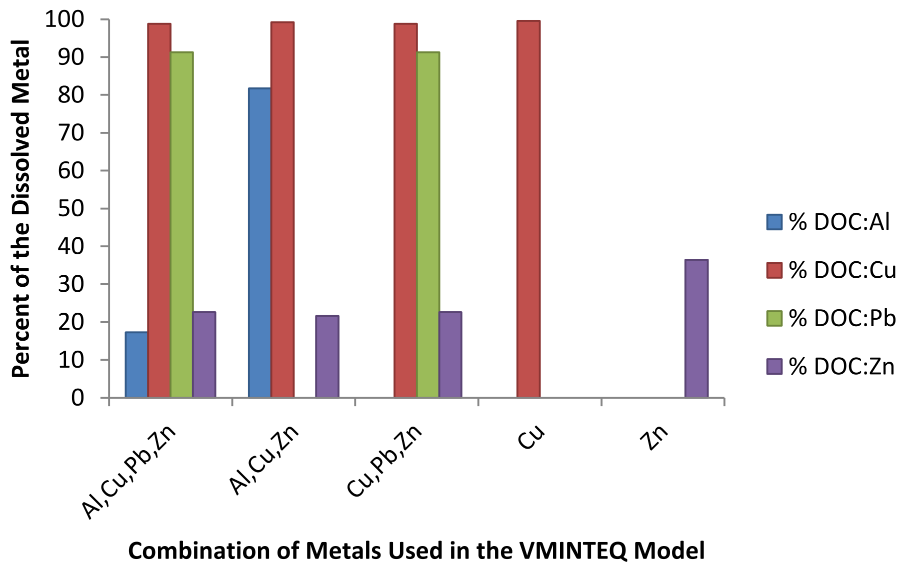


Figure 2: Percent of the toxic forms of Cu and Pb (Cu2+, CuOH+, and Pb2+) out of total dissolved species of that metal, calculated by VMINTEQ.

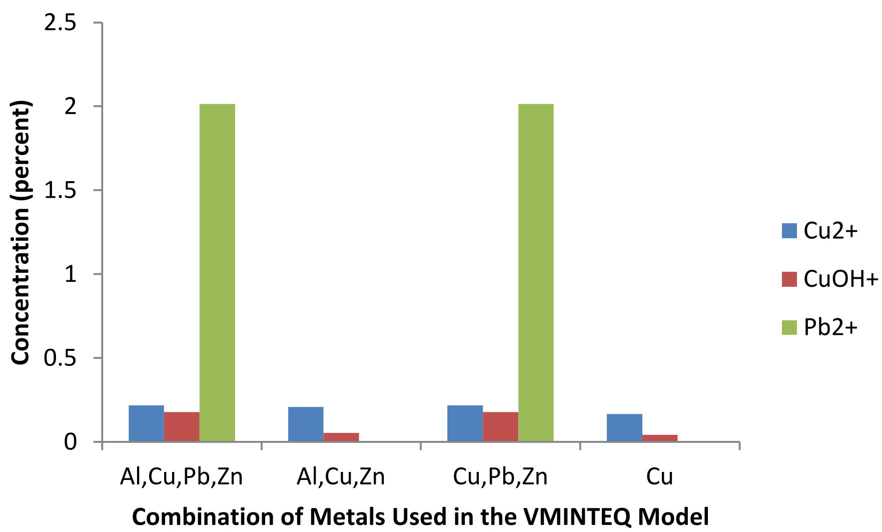
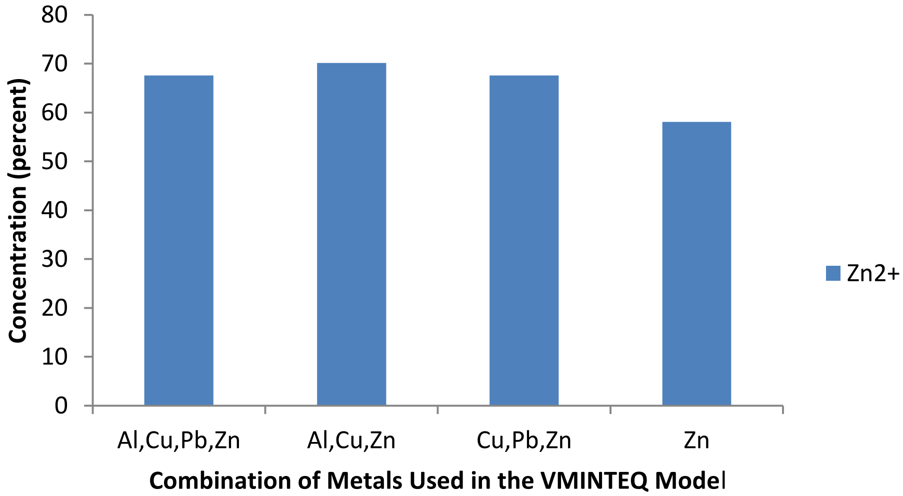


Figure 3: Percent of the toxic form of Zn (Zn^{2+}) out of the total dissolved Zn, calculated by VMINTEQ.



In summary, this modeling exercise demonstrates that the presence of multiple metals in a solution will influence which metals are complexed to humic and fulvic acids (Figure 1), and will therefore, also affect how much of the toxic form of the metal is present (Figures 2 and 3). It is important to note the limitations of this modeling exercise, which follow. It was not meant to reflect actual conditions in the Chuit River, rather the models were designed to show (relatively) how the presence of different metals can affect speciation, which will affect toxicity. Additionally, these models do not account for the coagulation and flocculation that could occur. Finally, how these metals exert toxicity as mixtures is not considered in this exercise.

5.4 Toxic Units Assessment of the Confirmatory Tests

An assessment of the Toxic Units was conducted to allow for a consistent comparison of individual WERs against the Confirmatory toxicity test results using the same hardness. Total Toxic Units (TTU) were calculated for the Confirmatory mixture test for *D. magna* and *P. promelas*. The dissolved and total metal concentrations measured in the site water from the Confirmatory tests were compared to the toxicity results from the WER tests using individual metal toxicity for each round in the site water (Equation 3) as described by USEPA (1991). As an additional comparison, the CMCs from the Alaska Water Quality Criteria (AWQC) were used (Equation 4). The Cu, Pb, and Zn AWQC and LC50s from the individual metals tests were hardness adjusted to 38 mg/L to match the water hardness of the site water in the Confirmatory test using equations in ADEC (2008) for AWQC corrections and Equation 5 for the LC50 corrections.

$$TTU_W = \frac{[Al]_c}{LC50_{Al}} + \frac{[Cu]_c}{LC50_{Cu}} + \frac{[Pb]_c}{LC50_{Pb}} + \frac{[Zn]_c}{LC50_{Zn}} \qquad \text{Equation 3}$$

where TTU_W is the Total Toxic Units normalized to the individual metal WER results; $LC50_x$ is the LC50 of metal x from the Tetra Tech (2010) WER (for example, $LC50_{Al}$ is the LC50 of Al); $[x]_c$ is the concentration of metal in the Confirmatory test (for example, $[Al]_c$ is the measured concentration of Al in the Confirmatory test) from Table 2 in Diamond and Latimer (2011).

$$TTU_A = \frac{[Al]_c}{CMC_{Al}} + \frac{[Cu]_c}{CMC_{Cu}} + \frac{[Pb]_c}{CMC_{Pb}} + \frac{[\mathrm{Zn}]_c}{CMC_{Zn}} \qquad \text{Equation 4}$$

where TTU_A is the Total Toxic Units normalized to the AWQC; CMC_x is the AWQC CMC of metal x from the WER (for example, CMC_{Al} is the CMC of Al).

$$LC50_{at\ hardness\ 1} = e^{\ln(LC50)-(slope \times (\ln(hardness\ 2)-\ln(hardness\ 1)))} \text{Equation 5}$$

where slope = 0.9422 for CU; 1.273 for Pb; and 0.8473 for Zn. From Tetra Tech (2010, Equation 2.1).

The LC50s were converted from total to dissolved (Al) and dissolved to total (Cu, Pb, and Zn) concentrations using the measured dissolved to total ratios in the site water from each round of testing (Table 4). The AWQC were hardness adjusted using the ADEC (2008) recommended Freshwater Conversion Factors.

Table 4: Summary of average dissolved to total ratio for all metals tested from the Chuitna individual metal WERs (Tetra Tech 2014).

Round	*Water*	*Species*	*Al*	*Cu*	*Pb*	*Zn*
1	*Lab*	*D. magna*	*0.04*	*0.86*	*0.93*	*0.98*
		P. promelas	*0.005*	*0.91*	*0.08*	*0.98*
	Site	*D. magna*	*0.06*	*0.87*	*0.62*	*0.84*
		P. promelas	*0.24*	*0.84*	*0.46*	*0.88*
2	*Lab*	*D. magna*	*0.08*	*0.92*	*1.21*	*1.05*
		P. promelas	*0.002*	*NA*	*NA*	*NA*
	Site	*D. magna*	*0.09*	*0.78*	*0.52*	*0.83*
		P. promelas	*0.19*	*NA*	*NA*	*NA*
3	*Lab*	*D. magna*	*NA*	*0.89*	*0.9*	*1.05*
		P. promelas	*0.02*	*NA*	*NA*	*NA*
	Site	*D. magna*	*NA*	*0.6*	*0.43*	*0.71*
		P. promelas	*0.53*	*NA*	*NA*	*NA*

NA - Not Available

Not all metals were used for all TTU calculations because: 1) In round three WER testing, *D. magna* were not exposed to Al; 2) In rounds 2 and 3 WER testing, *P. promelas* were not exposed to Cu, Pb, or Zn; and 3) A dissolved AWQC for Al does not exist. Excluding these metals results in lower TTUs than if all were included. In round 1 WER testing for Al and Pb and in round 2 WER testing for Al, the LC50 was greater than the highest concentration tested. The highest concentration tested, therefore, was used as the LC50 for those situations (e.g. in Al round 1 for *D. magna*, the LC50 was >190 mg/L so 190 mg/L was used as the LC0 in calculating the TTU_W). If an actual LC50 had been calculated, this would result in a lower TTU_W value since a larger number would be used in the denominator for that metal in Equation 3.

Assuming that these metals have additive toxicity, a TTU of 1 is interpreted as a mixture that would result in 50% mortality. A TTU less than 1 means the mixture should result in less than 50% mortality and a TTU greater than 1 means the mixture should result in more than 50% mortality. Results of the TTU are included as Tables 5 and 6. In all cases, the TTU_w is less than 1 and there was less than 50% mortality (no significant toxicity measured for either test species) in the Confirmatory tests as predicted by the TTU_W.

The TTU_A were all greater than 1. This is interpreted as a 4.34 to 23.05 factor[14] increase in the concentrations in the Confirmatory test compared to allowable (CMC) concentrations in a mixture. This TTU_A (for dissolved or total metals) provides a direct comparison to the CMCs from the AWQC. Different interpretations of the TTU_w or TTU_A calculated here would not be accurate.

Table 5: Total Toxic Units using dissolved metals concentrations

TTU type	Test species	WER Round	Metals Used for TTU	TTU
TTU_w	*D. magna*	1	Al,Cu,Pb, Zn	0.248
TTU_w	*D. magna*	2	Al,Cu,Pb,Zn	0.5
TTU_w	*D. magna*	3	Cu,Pb,Zn	0.26
TTU_A	*D. magna*	NA	Cu,Pb,Zn	4.34
TTU_w	*P. promelas*	1	Al,Cu,Pb,Zn	0.243
TTU_w	*P. promelas*	2	Al	0.005
TTU_w	*P. promelas*	3	Al	0.0005
TTU_A	*P. promelas*	NA	Cu,Pb,Zn	4.96

NA - Not Available

[14] These numbers are the minimum and maximum TTU_A from Tables 5 and 6.

Table 6: Total Toxic Units using total metals concentrations.

TTU type	Test species	WER Round	Metals Used for TTU	TTU
TTU_W	*D. magna*	1	Al,Cu,Zn	0.45
TTU_W	*D. magna*	2	Al,Cu,Pb,Zn	0.65
TTU_W	*D. magna*	3	Cu,Pb,Zn	0.41
TTU_A	*D. magna*	NA	Al,Cu,Pb,Zn	22.02
TTU_W	*P. promelas*	1	Al,Cu,Pb,Zn	0.08
TTU_W	*P. promelas*	2	Al	0.005
TTU_W	*P. promelas*	3	Al	0.002
TTU_A	*P. promelas*	NA	Al,Cu,Pb,Zn	23.05

NA - Not Available

6.0 Recommendations as they Relate to the Application of the WER

Two specific questions were posed by ADEC as part of this technical opinion.

1. *Is it appropriate to use the mixed metals Confirmatory tests to derive chronic criteria (as Region 10 EPA suggests) given the chemistry and precipitation problems associated with this test?*

   Application of the Confirmatory test to derive SSC is not recommended for the following reasons:

    a. It is not my interpretation that this is the purpose of the Confirmatory test as presented by the USEPA (1994) in Appendix F. This Appendix specifically deals with mixtures of metals when individual WERs are used to adjust the criteria to site specific conditions and states "If a WER is determined for each metal individually, one or more additional toxicity tests must be conducted at the end to show that the combination of all metals at their proposed new site specific criteria is acceptable." In my interpretation of this quote "at their proposed new site specific criteria…" indicates that the site specific criteria have been proposed prior to conducting the Confirmatory tests (using the single metals tests in the Chuit River case), so that modifying criteria again based on results of the Confirmatory test is not necessary or required.
    b. Based on the statistical analysis by Tetra Tech, there was no significant effect of the metals in the Confirmatory test. It cannot be determined how much more metal could be added to the site water before there would be a significant effect, which makes these concentrations overprotective to an unknown degree. As such, there is no basis to use these concentrations as the concentrations for site specific criteria calculations. If the intent is to

base the SSC on mixture testing, a different test design should be used where either: 1) a point estimate can be determined or 2) a NOEC/LOEC can be identified. The SSC could then be based on the concentration at which unacceptable effects occur.

c. The chemical speciation and resultant toxicity of these waters cannot be predicted using equilibrium models or with current knowledge of mixed metal toxicity, meaning that this Confirmatory test represents toxicity under only one set of conditions – it has no predictive ability. This is in part because the waters have not been adequately characterized for a more accurate representation of the metal speciation, but also because an accepted approach to predict mixture toxicity (with the exception of Toxic Units) does not exist and empirically collected data from other studies have shown that several factors affect whether metals behave additively, antagonistically, or synergistically (see Section 5.1). The use of an actual toxicity test (as conducted with the Confirmatory test), however, does eliminate the need to predict whether toxicity will occur since the organisms respond to the exact conditions they are exposed to.
d. Related to reason c, but more specifically, the Confirmatory tests used four metals (Al, Cu, Pb, and Zn). Site specific criteria is being requested for three metals (Al, Cu, and Zn). Based on the speciation of a theoretical water (Section 5.3) the presence of Pb affected the speciation of other metals in the Confirmatory test. This was an overly simplified model so exact effects cannot be definitively specified, but a change in speciation is expected in the absence or presence of Pb. If these metals are behaving additively, then the presence of Pb would result in an overprediction of Al, Cu, and Zn mixture toxicity. If the presence of the Pb results in chemical antagonism, then the Confirmatory tests would underpredict the toxicity of Al, Cu, and Zn in a mixture.
e. The Toxic Units calculations presented here indicate that mortality less than 50% was expected based on dissolved and total recoverable metals concentrations when normalized for lab water LC50s. As currently analyzed, there was no significant difference in mortality compared to the unspiked site water in the Confirmatory test. The concentration of metals used in Confirmatory tests exceeds the CMCs when the mixture of metals is considered. When metals are missing from any of the TTU calculations, the calculation should be considered a conservative one since more metals would result in a greater TTU. The greater the TTU, the more toxicity would be predicted.

f. The Confirmatory tests used one site sample, where the WER tests relied on three samples. A range of physicochemical conditions is not, therefore, considered with the Confirmatory test.
g. ADEC described the decreased solubility in the Confirmatory tests as "chemistry and precipitation problems." Instead, these should be considered as an example of the type of chemical reactions that would occur in natural waters. The goal of site specific criteria is to determine how site specific conditions influence toxicity; the site specific conditions include presence and concentrations of organic and inorganic ligands and other metals. In these waters, because of the chemical and precipitation reactions, dissolved concentrations were lower than predicted by USEPA (2009) and ADEC (2008) Correction Factors and significant toxicity was not measured at the total concentrations used in the Confirmatory tests. It was confirmed, therefore, that site specific conditions change the water quality such that toxicity occurs at different concentrations of metals than if laboratory water was used without the ligands or other metals present.
h. Finally, the Confirmatory tests did not advance the understanding of mechanisms through which toxicity would occur with multiple metals, nor did it advance the understanding of the changes to metal speciation. Without a better understanding of these, there is little scientific evidence to support changing the SSC derived from the WERs to a SSC derived from the Confirmatory tests.

The second question posed by ADEC was:

2. *Does the methodology allow the use of individual WER tests to derive the site specific criteria, even if the mixed metals Confirmatory tests did not have [dissolved] metals concentrations as high as the individual WER tests?*

   It is my opinion that the individual WER tests are the best methodology available to derive the SSC. As described above, the Confirmatory test supported that there was no significant toxicity at the total metals concentrations used which were based on the individual WER tests. The fact that dissolved concentrations could not be achieved as predicted by USEPA and ADEC Correction Factors is a consequence of complicated aqueous and surface chemistry and is representative of what would happen in natural (Chuit River) waters.

> Finally, my conclusion based on the analysis presented in this report is that the presence of Al is likely to result in chemical antagonism since dissolved Cu and Zn concentrations should and did decrease.

As opposed to using the Confirmatory results to derive site specific criteria, I recommend that the site specific criteria be derived for Al (as total recoverable) using the single metal WER for the following reasons:

1. ADEC (2008) supports that a SSC based on a WER is appropriate for Al.

2. ADEC (2008) summarize a study with brook trout exposed to Al at low pH and hardness. Toxic effects increased as total Al increased despite dissolved Al remaining constant (ADEC 2008). This supports that a mechanical mechanism (as opposed to a chemical mechanism caused this toxicity, see Section 5.2.1) and SSC based on total recoverable should account for this mechanism of toxicity, although it is likely to be overprotective as described by Butcher (1988).
    a. Al is often associated with clay and other secondary minerals which are biounavailable and would decrease toxicity; these would be measured with total recoverable Al which adds a level of conservatism to this criteria (Butcher 1988, ADEC 2008).

3. As of now, there is no consensus as to how Al should be regulated as a dissolved fraction.

Finally, I recommend that Cu and Zn criteria be derived (as dissolved) using the individual WERs for those metals. Copper and Zn criteria are currently regulated as dissolved concentrations because a scientific consensus exists as to how these metals exert toxicity in freshwater systems (BLM). There is no scientific reason to change how these metals are currently regulated based on the results of the Confirmatory test.

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Appendix A: Clarifications on Confirmatory Test from Tetra Tech 2014

Tetra Tech (Tt) Response to Ruth Sofield Inquiries

1) Sofield: It looks like the lab water was soft water (according to the CETIS reports). Can you confirm that is correct and that the soft water recipe from ASTM or WET was used? (i.e. 48 mg/L NaHCO3, 30 mg/L CaSO4 2H20, 30 mg/L MgSO4 and 2 mg/L KCl).

Tt Response: Laboratory dilution water for copper, lead, and zinc was reconstituted using EPA's formulation (EPA 2002) to simulate the expected hardness of the site water. The dilution water consisted of reconstituted water with hardness between 22 mg/L and 26 mg/L (Table 1). EPA (2002) presents preparation methodology for "very soft" and "soft". The "very soft" preparation methodology was used for the reconstituted water used for aluminum testing. Because the "soft" preparation methodology would have an approximate hardness of 40 – 48 as indicated by USEPA (2002a), the preparation methodology used was between the "very soft" and the "soft" which resulted in a hardness of 20 – 25 mg/L as $CaCO_3$. This water was used for copper, lead, and zinc WER testing.

Table 1. *Amount of constituents added to make either ultra-soft (hardness 10 – 13 mg/L as $CaCO_3$) or soft (hardness 20 – 25 mg/L as $CaCO_3$).*

Round	*Metal*	*NaHCO3 (mg/L)*	*MgSO4 (mg/L)*	*KCl (mg/L)*	*CaSO4*2H2O (mg/L)*
1	*Al*	*12.0*	*7.5*	*0.51*	*7.5*
	Cu, Pb, Zn	*30.0*	*18.6*	*1.28*	*18.8*
2	*Al*	*12.0*	*7.5*	*0.52*	*7.5*
	Cu, Pb, Zn	*30.0*	*18.8*	*1.25*	*18.8*
3	*Al*	*12.0*	*7.5*	*0.52*	*7.5*
	Cu, Pb, Zn	*30.0*	*18.8*	*1.28*	*18.8*

Sofield: No response necessary.

Clarifying Questions:

Sofield: On the attached document[15], page 2, there is a statement as follows:
"In keeping with using total metals in confirmatory testing, we would herein propose that site specific
criteria for copper, zinc, and lead be based on total recoverable rather than dissolved standards; i.e., the WERs determined in this study would be applied on the basis of total recoverable rather than dissolved copper, lead, and zinc. This would add an additional measure of conservatism to ensure that the site-specific criteria are protective of aquatic life in the Chuitna."

[15] Diamond and Latimer, 2010a.

2) Sofield: Are you recommending that the WERs from the individual tests be applied to the total metals from the ADEC criteria (which are dissolved and would need to be converted to total using which dissolved:total ratio - ADEC's or yours from single tests or yours from mixed test?) or that the WERs from the individual tests be applied to the total metal concentrations used in the mixture (confirmatory) test or that the WERs from the individual tests be applied to the total metals in the single metal tests or something else?

Tt Response: ADEC criteria for copper, lead, and zinc, which are based on dissolved, can be converted to total based on the conversion factors (CF) provided by USEPA in the National Recommended Aquatic Life Water Quality Criteria (USEPA 2002b) and ADEC Water Quality Criteria Manual for Toxic and Other Deleterious Organic and Inorganic Substances (ADEC, 2008). Conversion factors for acute and chronic criteria for copper, lead, and zinc are very close to 1.00 as shown below:

Table 2. *Summary of total to dissolved conversion factors from USEPA (2002) and ADEC (2008).*

Parameter	*Acute Conversion Factor*	*Chronic Conversion Factor*
Copper	*0.960*	*0.960*
*Lead**	*1.46203-[(ln hardness)(0.145712)]*	*1.46203-[(ln hardness)(0.145712)]*
Zinc	*0.978*	*0.986*

**= 0.993 at 25 mg/L as $CaCO_3$ hardness*

These conversion factors (CF) are used in the equations provided by USEPA (2002) and ADEC (2008) to calculate the dissolved metals criteria from the total metal criteria:

$$CMC\ (dissolved) = exp\{m_A[ln(hardness)]+ b_A\}(CF)$$

$$CCC\ (dissolved) = exp\{m_C[ln\ (hardness)]+b_C\}(CF)$$

Where m_A, b_A, m_C, and b_C are defined in the documents and CF is the conversion factor listed above.

<u>Sofield</u>: My interpretation of this answer is that the ADEC WQC as dissolved would be converted to total, and the WER applied to that concentration. This was confirmed by personal communication with Bowersox and Diamond (Tetra Tech) on 6/25/14.

3) Sofield: Can you explain the thinking behind why this would provide more conservatism?

Tt Response: Basing the WERS and site-specific criteria on total rather than dissolved metal is conservative because the total metal concentration will always be greater than the dissolved fraction. Thus, if the WER is applied to the total metal concentration and the dissolved metal concentration (which is the biologically active portion) is actually only a portion of the total, then the site specific criteria would be conservative. If one applied the WER to the dissolved fraction and the dissolved fraction is actually less than

100% of the total, then one could conceivably discharge more metal as total metal. For example, assume that the WER for copper, based on dissolved copper, indicated a criterion of 60 µg/L dissolved copper. If the dissolved fraction was only 50% of the total metal measured then 120 µg/L total copper could be discharged, but if the criterion was expressed as total, then in this example only 30 µg/L dissolved metal would be actually allowed (total = 60 µg/L).

Sofield: Confirmed with Bowersox and Diamond (personal communication, 6/25/14) that the ADEC/EPA Correction Factor is used (which is my interpretation of the previous answer)

4) Sofield: In the same document, there is a statement "The site-specific acute and chronic criteria for copper, lead, and zinc differ only slightly when represented as the total recoverable fraction instead of the dissolved fraction (less than 4% difference depending on the metal)."

Is this calculation (4% difference) based on a) running CETIS with the total metal concentrations and with the dissolved concentrations and comparing those LC50s or by taking the dissolved LC50s and applying some correction factor for dissolved to total (if so, what is that correction factor)?

Tt Response: See response above for conversion factors that account for less than 4% difference in total recoverable versus dissolved fraction for copper, lead, and zinc.

Sofield: I interpret this to mean that CETIS uses dissolved Cu, Pb, and Zn concentrations or total Al concentrations, which were converted with the ADEC CF to total Cu, Pb, and Zn (no conversion for Al in ADEC WQC). However, the sentence that follows the statement in question is: "In addition, the total to dissolved ratios for these metals in the ADEC water quality standards are also near 1.0, indicating little difference between total and dissolved standards for these metals." Meaning this second statement in Tetra Tech (2010) is redundant since the previous sentence in that document uses the same ADEC dissolved to total ratios that the second sentence refers to.

The requests are:

5) Sofield: Can you provide the dissolved to total metal ratios for each round of individual tests?

Tt Response: See Table below for the calculated dissolved to total ratio for each contaminant and each round of WER testing. Ratio presented is the average ratio for lab or site water. Ratios were calculated by dividing the measured dissolved fraction by the measured total fraction for each concentration that was measured.

Table 3. *Summary of average total:dissolved ratio for all metals tested for Chuitna WER.*

Round	*Water*	*Species*	*Al*	*Cu*	*Pb*	*Zn*
1	*Lab*	*D. magna*	*0.04*	*0.86*	*0.93*	*0.98*
		P. promelas	*0.005*	*0.91*	*0.08*	*0.98*
	Site	*D. magna*	*0.06*	*0.87*	*0.62*	*0.84*
		P. promelas	*0.24*	*0.84*	*0.46*	*0.88*
2	*Lab*	*D. magna*	*0.08*	*0.92*	*1.21*	*1.05*
		P. promelas	*0.002*			
	Site	*D. magna*	*0.09*	*0.78*	*0.52*	*0.83*
		P. promelas	*0.19*			
3	*Lab*	*D. magna*		*0.89*	*0.90*	*1.05*
		P. promelas	*0.02*			
	Site	*D. magna*		*0.60*	*0.43*	*0.71*
		P. promelas	*0.53*			

<u>Sofield</u>: No response necessary.

6) Sofield: Can you explain how those ratios were calculated? Is it the average of the ratio for all test concentrations or something else?

Tt Response: Ratios were calculated by dividing the measured dissolved fraction by the measured total fraction for each concentration that was measured. Total and dissolved metal concentrations were measured at test initiation and dissolved metal concentration was measured prior to renewal at 24 hours or prior to test completion at 48 hours, if a renewal at 24 hours was not necessary. The ratios presented are the average ratio (both total:initial dissolved and total:final dissolved) for lab or site water for all concentrations measured per round.

<u>Sofield</u>: Good approach.

7) Sofield: If you calculated the LC_{50} for Cu, Pb, and Zn using total metal concentrations and the LC_{50} for Al as dissolved concentrations, can you make those results available?

Tt Response: These LC50s were not calculated in this study as they were not required. However, based on the total to dissolved ratio of nearly 1.0 (see tables presented above), the LC_{50}s for total copper, lead, and zinc are expected to be similar to LC_{50}s based on the dissolved fraction.

<u>Sofield</u>: No response necessary.

8) Sofield: If you did any range finding type of tests with the mixtures, could you make those details (concentration series and results) available?

Tt Response: The testing completed for the analysis of the water effects ratio (WER) were used as range finding tests to determine the appropriate concentrations used in the mixture tests. No additional range-finding tests were conducted.

Sofield: from EPA (Beckwith 2013), "A third point concerning additional information related to the metals mixture test; a 12/14/10 email from Dan Graham of PacRim indicated that Tetra Tech ran a series of tests spanning the target level for the WER confirmation tests. Both ADEC and EPA had suggested such testing because it could be useful in evaluating the effects of the metals in combination." leads me to believe that EPA expected range finding type tests, which did not occur. Bowersox and Diamond (personal communication, 6/25/14) did state that some preliminary tests were conducted to assess solubility of the metals only (no toxicity was assessed with these preliminary tests).

9) Sofield: Can you provide the water quality measurements for the Confirmatory tests? Most important is the hardness that test was conducted at, but other parameters (including DOC) would be useful.

Tt Response: The water quality including hardness, alkalinity, dissolved oxygen, pH, conductivity, ammonia, chlorine, dissolved organic carbon (DOC), total organic carbon (TOC), and total suspended solids (TSS) of the site water that was used in the confirmatory testing is included in the table below.

Table 4. *Summary of water quality from the Chuitna site water used in confirmatory testing.*

Parameter	*Range for D. magna Testing*		*Range for P. promelas Testing*	
	Minimum	*Maximum*	*Minimum*	*Maximum*
Dissolved Oxygen (mg/L)	*8.7*	*10.4*	*8.1*	*10.1*
pH (su)	*6.9*	*7.8*	*6.9*	*7.8*
Conductivity (umhos/cm)	*77.3*	*85.9*	*76.8*	*81.3*
Temperature (°C)	*24.3*	*24.4*	*24.3*	*24.4*
Ammonia (mg/L)	*0.03*			
Chlorine (mg/L)	*ND (<0.01)*			
Hardness (mg/L as CaCO3)	*38*			
Alkalinity (mg/L as CaCO3)	*14*			
DOC (mg/L)	*4.3*			
TOC (mg/L)	*4.9*			
TSS (mg/L)	*46*			
Total Al (mg/L)	*0.21*			
Total Cu (mg/L)	*ND (<0.001)*			
Total Pb (mg/L)	*ND (<0.001)*			
Total Zn (mg/L)	*ND (<0.005)*			

Sofield: No response necessary

10) Sofield: From Tables 2 and 3 (Jan 2011 memo)[16] on the attached file, can you clarify if the measured total recoverable metals in the spiked site are the concentrations measured in the spike before dilution or in the 80% dilution used in the toxicity tests?

Tt Response: The measured total recoverable and measured dissolved metal concentrations presented in Tables 2 and 3 are the average concentrations measured in the 80% dilution used in the toxicity testing. Total recoverable and dissolved metal concentration was measured at test initiation, before test renewal at 24 hours, after test renewal at 24 hours, and at test completion at 48 hours. The average of these four values is what is presented in Tables 2 and 3 of the Jan 2011 memo.

Sofield: No response necessary.

11) Sofield: From those same Tables (2 and 3), can you clarify how the total recoverable acute criterion was calculated? I believe it is hardness corrected, but can't be sure. I also can't tell what Dissolved:Total conversion was used.

Tt Response: The "Total Recoverable Acute Criterion" was calculated based on the site hardness of 38 mg/L. The dissolved:total conversion used was what is presented by USEPA (2002) and ADEC (2008) and summarized in Table 2 above.

Sofield: No response necessary.

References used by Tetra Tech (2014) for their responses:

U.S. Environmental Protection Agency. 2002a. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition, October 2002. EPA-821-R-02-012. Office of Water, Washington, DC.

U.S. Environmental Protection Agency (USEPA). 2002b. National Recommended Water Quality Criteria: 2002. EPA-822-R-02-047. Office of Water, Washington, DC.

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[16] Diamond and Latimer 2011.

Appendix B: Abbreviated CV for Sofield

Ruth M. Sofield (Harper)

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EDUCATION

Doctorate of Philosophy in Environmental Science and Engineering with a Minor in Management, May 2002
Colorado School of Mines, Golden, CO
Dissertation Title: Genetically Based Tolerance to Chemical Exposure in the Grass Shrimp, *Palaemonetes pugio*.
Research Advisor: Dr. Philippe Ross, Colorado School of Mines

Master of Science in Environmental Science and Engineering, December 1999
Colorado School of Mines, Golden, CO

Master of Science in Environmental Science, May 1995
McNeese State University, Lake Charles, LA

Bachelor of Arts in Biology, May 1993
West Virginia University, Morgantown, WV ü

SELECTED WORK EXPERIENCE

Associate Professor, Department of Environmental Sciences, Huxley College of the Environment, Western Washington University**,** 2009 – present

Assistant Professor, Department of Environmental Sciences, Huxley College of the Environment, Western Washington University**,** 2003 – 2009

Marine and Estuarine Science Program Faculty, Shannon Point Marine Center, 2004 – present

Post-doctoral Fellow, Colorado School of Mines, 2002 – 2003

Field Biologist, Center for Coastal Environmental Health and Biomolecular Research, Marine Ecotoxicology Branch, National Ocean Service, NOAA, Charleston, SC, 1999 – 2002

Marine Ecotoxicology Teaching Assistant, June 2001 – July 2001
The Bermuda Biological Station for Research, Inc.

Toxicology Investigator, July 2000 – May 2002
Investigation of acute toxicity of mine tailings leachate to *Daphnia magna, Vibrio fischeri,* and *Lactuca sativa* using standard testing procedures, including ASTM and Microtox protocols.

SELECTED RESEARCH

Environmental Chemistry and Toxicity of Silver Nanoparticles, Visiting Researcher, Work conducted at Environmental Toxicology Department at EAWAG in Dübendorf, Switzerland. September 2010 – August 2011.

Texas A&M at Galveston. End goal is to apply knowledge to possible bioremediation of radionuclide contaminated areas.

Genetically Based Tolerance to Chemical Exposure in the Grass Shrimp, *Palaemonetes pugio*, Ph.D. Dissertation Research, January 1999 – May 2002
Primarily funded by the National Ocean Service, NOAA, Charleston, SC.
Investigation of whether *P. pugio* (grass shrimp) that are tolerant to chemical (endosulfan, chromium, and fluoranthene) exposure have different allozyme frequencies than grass shrimp that are not tolerant to chemical exposure.
Research included "Time to Death" tests of adult *P. pugio*, hatchability and development of *P. pugio* embryos, allozyme analysis, and computer modeling of effects to *P. pugio* populations.

Hydrologic and Geochemical Functions of Southern Rocky Mountain Wetlands,
May 1997 – May 1999
Funded by Colorado Geologic Survey and Colorado Scientific Society.
Investigated wetland functions; involved characterization of wetland hydrology and wetland biogeochemistry.

RESEARCH PUBLICATIONS

Smith K, Ranville J, Lesher E, Diedrich D, McKnight D, **Sofield R**. Fractionation of Dissolved Organic Matter by Iron and Aluminum Oxides—Influence on Copper Toxicity to *Ceriodaphnia dubia*. Submitted to *Environmental Science and Technology* (in review).

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Sofield RM. 2013. A Pilot Investigation of the Pollution and Toxicity of Snowpack Collected from Snowmobile Recreation Areas in Washington and Wyoming. Final Report: Submitted to Winter Wildlands Association.

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Martins M, Bollinger C, **Harper R**, Ribeiro R. 2009. Effects of Acid Mine Drainage on the Genetic Diversity and Structure of a Natural Population of *Daphnia longispina*. *Aquatic Toxicology*. 92:104-112.

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Harper RM, Kantar C, and Honeyman BD. 2008. Binding of Pu (IV) to Galacturonic Acid and Extracellular Polymeric Substances (EPS) from *Shewanella putrefaciens*, *Clostridium* sp., and *Pseudomonas fluorescens*. *Radiochimica acta*. 96:753-762.

Harper RM, Tinnacher R. 2008. Ecotoxicology: Plutonium. Sven Erik Jorgensen & Brian D. Fath (Eds.), Encyclopedia of Ecology, 1st Edition, Elsevier B.V., Oxford, pp 2845- 2850.

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Martins N, Lopes I, **Harper R**, Ross P, and Ribeiro R. 2007. Differential Resistance to Copper and Mine Drainage in *Daphnia longispina*: Relationship with Allozyme Genotypes. *Environmental Toxicology and Chemistry. 26(9):1904-1909.*

Kantar C, Gillow JG, **Harper-Arabie RM,** Honeyman BD, and Francis AJ. 2005. Determination of Stability Constants of U(VI)-Fe(III)-Citrate Complexes. *Environmental Science and Technology*. 39(7): 2161-2168.

Harper-Arabie RM, Wirth EF, Fulton MF, Scott GI, and Ross PE. 2004. Protective Effects of Allozyme Genotype During Chemical Exposure in the Grass Shrimp, *Palaemonetes pugio. Aquatic Toxicology.* 70(1): 41-54.

Harper-Arabie RM, and Kolm KE. An Integrated Approach to Wetlands Characterization in Determining Hydrologic Functions. *In*: The First International Symposium on Integrated Technical Approached to Site Characterization, Argonne National Laboratory, Illinois, 1998.

Kolm KE, **Harper-Arabie RM**, and Emerick JC. 1998. Chapter *In* Final Report: Characterization and Functional Assessment of Reference Wetlands in Colorado; A Preliminary Investigation of Hydrogeomorphic (HGM) Classification and Functions for Colorado's Wetlands. Colorado Department of Natural Resources and U.S. EPA, Region VIII, Denver, CO.

BOOKS

Landis WG, **Sofield RM**, and Yu MH. 2010. Introduction to Environmental Toxicology: Impacts of Chemicals Upon Ecological Systems. 4th Edition.

OTHER PUBLICATIONS

Chair of revisions for Standard Methods 8050: Bacterial Bioluminescence Test, 23rd Edition.
Chair of revisions for Standard Methods 8711: *Daphnia*, 23rd Edition.
Chair of revisions for Standard Methods 8050: Bacterial Bioluminescence Test, 22nd Edition.
Chair of revisions for Standard Methods 8711: *Daphnia*, 22nd Edition.

SELECTED PRESENTATIONS

Johnson J, Kelley A, and **Sofield RM**. 2014 Lichens as Air Quality Biomonitors Along the Pacific Northwest Rail Corridor in Bellingham, Washington. Pacific Northwest SETAC Annual Meeting, Tacoma, WA.

San C, Fung C, **Sofield RM**. 2014. Investigation of Silver Nanoparticle Toxicity at Different Temperatures. Pacific Northwest SETAC Annual Meeting, Tacoma, WA.

Fix JE, and **Sofield RM**. 2013. Investigation of Biochemical Responses of Lichens to Air Pollutants Originating from trains in Northwestern Washington. Pacific Northwest SETAC Annual Meeting, Spokane, WA.

Sorensen TM, and **Sofield RM**. 2013. Silver Concentration Detection with modified Environmental Factors Using Ion Selective Electrodes. Pacific Northwest SETAC Annual Meeting, Spokane, WA.

Eckard SM, and **Sofield RM**. 2013. Analysis of Pollutants in the High Alpine Aquatic Systems of the Cordillera Blanco Mountain Range of Central Peru. Pacific Northwest SETAC Annual Meeting, Spokane, WA.

Sofield RM, Wagner B, Sigg L, and Behra R. 2012. Interactions of Humic and Fulvic Acids with Silver Nanoparticles and the Resultant Toxicity to *Chlamydomonas reinhardtii*. SETAC North America Meeting, Long Beach, CA.

Combs R, Murdock C, and **Sofield RM**. 2012. The Influence of Capping Agent, Ion Concentration, and Humic Acids on the Toxicity of Silver Engineered Nanoparticles. SETAC North America Meeting, Long Beach, CA.

Crowell T, Murdock C, Ford J, and **Sofield RM**. 2012. Modifying Effects of Temperature on Metal Toxicity to *Lemna turionifera*. SETAC North America Meeting, Long Beach, CA.

Gibson AJ, and **Sofield RM**. 2012. Acute and Chronic Toxicity of Ag Nanoparticles to *Daphnia magna* through Aquatic and Ingestion Routes of Exposure. SETAC North America Meeting, Long Beach, CA.

Lowery G, Patmont E, and **Sofield RM**. 2012. Modeling Bioaccumulation of Contaminants in Puget Sound: An Analysis of Site-Specific Parameters. SETAC North America Meeting, Long Beach, CA.

Ragsdale R, **Sofield R**, Bousquet T, Streblow B, Cook D, Stratton S, Ikoma J, and Finders C. 2012. Evaluating the Contribution to Toxicity of Weak Black Liquor in Bleached Kraft Pulp Mill Effluents. SETAC North America Meeting, Long Beach, CA.

Stiles J, and **Sofield RM**. 2012. An Analysis of the Risks and Benefits of Fish Consumption in the Pacific Northwest. SETAC North America Meeting, Long Beach, CA.

Sigg L, Piccapietra P, Lindauer U, Odzak N, **Sofield R**, and Behra R. 2012. Silver Nanoparticle Dissolution and Ag Speciation as Key Parameters for Toxicity of AgNP to Algae. 6th SETAC World Congress / SETAC Europe 22nd Annual Meeting, Berlin, Germany.

Combs RD, Murdock CL, and **Sofield RM**. 2012. Toxicity of Silver Nanoparticles with Three Capping Agents (PVP, Citrate, and CO_3): An Evaluation of Ionic versus Total Silver. Pacific Northwest SETAC Annual Meeting, Vancouver, BC.

Ford JM, Crowell TM, and **Sofield RM**. 2012. Influence of Temperature on Heavy Metal Toxicity to *Lemna turionifera*. Pacific Northwest SETAC Annual Meeting, Vancouver, BC.

Wood D, Bergmann A, Combs RD, **Sofield RM**, and Church B. 2012. Contamination and Toxicity of Snowpack Collected from Snowmobile Recreation Areas. Pacific Northwest SETAC Annual Meeting, Vancouver, BC.

Heimbigner D, and **Sofield RM**. 2010. Fungal Growth Inhibition Bioassay Using Silver Nanoparticles. SETAC North America Annual Meeting, Portland, OR.

Church B, and **Sofield RM**. 2010. Ability of the white rot fungus *Pleurotus ostreatus* to degrade benzo[*a*]pyrene under variable surfactant amendment regimes. SETAC North America Annual Meeting, Portland, OR.

Ferguson M, and **Sofield RM**. 2010. Evaluating the Partitioning of Silver Nanoparticles in Aqueous Media Using the Freshwater Alage *Pseudokirchneriella subcapitata*. Pacific Northwest SETAC Annual Meeting, Port Towsend, WA.

Duncanson E, and **Sofield RM**. 2010. Community Level Effects on Benthic Macroinvertebrates from Exposure to Metals in Mine Impacted Creeks in Idaho. Pacific Northwest SETAC Annual Meeting, Port Towsend, WA.

Smith KS, Ranville JF, Diedrich DJ, McKnight DM, and **Sofield RM**. 2010. Influence of Organic-Matter Fractionation by Natural Iron Nanoparticles on Copper Speciation and Aquatic Copper Toxicity. American Chemical Society Spring 2010 National Meeting and Exposition. San Fancisco, CA. Abstract Submitted.

Smith KS, Ranville JF, Diedrich DJ, McKnight DM, **Sofield RM**. 2010. Influence of Dissolved Organic Matter in Determining Aquatic Copper Toxicity in Iron-Rich Environments. Seventh National Monitoring Conference National Water Quality Monitoring Council (NWQMC), Denver, CO. Abstract Submitted.

Smith KS, Ranville JF, Diedrich DJ, McKnight DM, **Harper RM**. 2009. Consideration of Iron-Organic Matter Interactions when Predicting Aquatic Toxicity of Copper in Mineralized Areas. 8^{th} ICARD (International Conference on Acid Rock Drainage), Skellefteå, Sweden.

Honeyman BD, Tinnacher R, Diaz A, Kantar C, **Harper R**, Gillow J. 2009. Biogeochemistry of Pu Transport. American Chemical Society Annual Meeting, Salt Lake City, UT.

Plante J, Bollinger C, Lenaker P, **Harper RM**. 2008. Development of a Benthic Index to Assess Metals Contamination Associated with Mining Waste in Canyon Creek (Coeur d' Alene). Pacific Northwest SETAC Annual Meeting, Corvalis, OR.

Fortner JC, **Harper RM**, Sternberg D. 2008**.** Shedding Light on Toxicity Testing – UV Light and PAH-Contaminated Groundwater. Pacific Northwest SETAC Annual Meeting, Corvalis, OR.

Fortner JC, **Harper RM**, Sternberg D. 2007. Using WET Test Methods to Detect Phototoxic Effects in PAH-Contaminated Groundwater. Society of Environmental Toxicology and Chemistry (SETAC) 28th Annual North America Meeting, Milwaukee, WI.

Honeyman B, **Harper R**, Kantar C, Moran P. 2007. The Complexation of U(Vi), Pu(Iv) and Np(V) With Natural Organic Ligands Using the Affinity Distribution Approach. *Migration 2007*, 11th Conference. Munich, Germany.

Harper R, Bollinger C. 2007. Evaluating the Effects of Metal Contamination on Aquatic Environments using Laboratory Assays, Field Assessments, and Chemical Models. Invited talk to *Joint Seminar on Environmental Science and Disaster Mitigation 2007*, Muroran, Hokkaido, Japan.

Fortner JC, **Harper RM**, Sternberg D. 2007. Phototoxicity in Whole Effluent Toxicity Testing: Lighting Considerations. Pacific Northwest SETAC Annual Meeting, Port Townsend, WA.

Stoddard JL, **Harper RM**. 2007. Effects of Multi-Generational Acclimation on the Toxicity of Copper, Cadmium, and Zinc to *Daphnia magna*. Pacific Northwest SETAC Annual Meeting, Port Townsend, WA.

Bollinger C, **Harper R**. 2007. Metal Speciation and Toxicity in Upper Columbia River and the Tributaries along the U.S. – Canadian Border using the Biotic Ligand Model and VMINTEQ. Pacific Northwest SETAC Annual Meeting, Port Townsend, WA.

Bollinger C, **Harper R.** 2006. Assessment of Metal Speciation and Toxicity in Upper Columbia River and the Tributaries along the U.S. – Canadian Border using the Biotic Ligand Model and VMINTEQ. 33rd Annual Aquatic Toxicity Workshop, Jasper, AB, Canada.

Bollinger C, **Harper R.** 2006. Population and Community Level Effects of Macroinvertebrates in Mining Impacted Streams. SETAC Europe 16th Annual Meeting, The Hague, The Netherlands.

Harper-Arabie, RM. 2005. Environmental Toxicology: Metals, Genes, Enzymes, and Size. Invited talk presented to the Biology Department, University of Aveiro, Portugal.

Diedrich D, Ranville J, Ross P, **Harper-Arabie R**, Brinkman S, Hoff D, and Wall D. Zinc toxicity to Brown Trout of various chronological ages and stages of acclimation. Society of Environmental Toxicology and Chemistry (SETAC) 26th Annual North America Meeting, Baltimore, MD 2005.

Bollinger C, **Harper-Arabie R.** 2005. AFLP analysis of *Hyalella azteca* collected from metals contaminated sediments. Society of Environmental Toxicology and Chemistry (SETAC) 26th Annual North America Meeting, Baltimore, MD.

Diedrich DJ, Ranville, JF, Ross PE, **Harper-Arabie, RM.** 2005. Zinc Toxicity to Brown Trout of Various Chronological Ages. Pacific Northwest SETAC Annual Meeting, Port Townsend, WA.

Diedrich DJ, Brinkman S, Ranville,JF, Ross,PE, **Harper-Arabie RM**, Hoff DJ, Wall DV. 2005. The Effects of Chronological Age on the Toxicity of Zinc to Brown Trout (*Salmo trutta*). Rocky Mountain SETAC Annual Meeting, Leadville, CO.

Harp AM, and **Harper-Arabie RM**. 2004. Effects of Water Chemistry on Metal- Metal Interaction in *Daphnia magna*. Society of Environmental Toxicology and Chemistry (SETAC) 25th Annual North America Meeting, Portland, OR.

Harper-Arabie RM, and Landis WG. 2004. Environmental Toxicology at Western Washington University. Invited speaker at SETAC 25rd Annual North America Meeting, Portland, OR.

Harper-Arabie RM, Hung CC, Buckley P, and Honeyman BD. 2004. Interactions of Microbial Exopolysaccharides with Plutonium in the Subsurface Environment. Pacific NorthWest SETAC Annual Meeting, Port Townsend, WA.

Martins N, **Harper-Arabie RM**, Lopes I, Ross PE, and Ribeiro R. 2003. Adaptation of Starch Gel Electrophoresis Techniques for Allozyme Analysis in Two Natural Populations of *D. longispina*. 5th Iberian Congress and 2nd Iberoamerican on Environmental Contamination and Toxicology Annual Meeting, Porto, Portugal.

Honeyman BD, **Harper-Arabie RM**, Francis AJ, Gillow JG, Dodge CJ, Santschi PH, and Hung CC. Plutonium Organic Complexes in the Environment: Stability and Biodegradation. 2003. Migration Conference, Korea.

Harper-Arabie R. M., Honeyman B. D. 2003. Effects of Organic Ligands on Uranium Speciation and Implications to Microbial Stabilization of Uranium. Conference Proceedings. Uranium Geochemistry 2003 Conference. Nancy, France.

Harper-Arabie RM, Fulton M, Wirth EF, and Ross PE. 2002. Reproductive Effects of Metals Exposure to *Palaemonetes pugio*: Influence of Genetics. SETAC 23rd Annual North America Meeting, Salt Lake City, UT.

Manock JJ, Wells PG, Owen RJ, Depledge ME, and **Harper-Arabie RM**. 2002. Marine Ecotoxicology Course at the Bermuda Biological Station for Research (1978-2002). SETAC 23rd Annual North America Meeting, Salt Lake City, UT.

Smith KS, **Harper-Arabie RM,** and Ross PE. 2002. Toxicity of Mine-Waste Leachates to Aquatic Organisms as a Function of pH and Trace-Metal Concentrations. Geological Society of America Annual Meeting, Denver, CO.

Harper-Arabie RM, Wirth EF, Fulton M, and Ross PE. 2002. Reproductive Effects of *In situ* Chromium Exposure to *Palaemonetes pugio* (grass shrimp). SETAC, Europe 12th Annual Meeting, Vienna, Austria .

Harper-Arabie RM, Ross PE, and Wirth EF. 2001. Genetic Basis of Tolerance in *Palaemonetes pugio* (grass shrimp) exposed to endosulfan, chromium, and fluoranthene. SETAC 22nd Annual North America Meeting, Baltimore, MD.

Harper-Arabie RM, Smith KS, and Ross PE. 2001. Toxicity of Metal Laden Leachate from Mining Waste Dumps to *Daphnia magna* and *Vibrio fischeri*. SETAC 22nd Annual North America Meeting, Baltimore, MD.

Harper-Arabie RM, Ross PE, and Wirth EF. 2001. Genetically Based Tolerance to Toxicant Exposure in *Palaemonetes pugio* (grass shrimp). Rocky Mountain SETAC Chapter Meeting**,** Denver, CO.

Harper-Arabie RM, and Kolm KE. 1999. A Hydrologic Analysis of Wetlands in the Southern Rocky Mountains of Colorado. American Geophysical Union, San Francisco, CA.

Kolm KE, Glover KC, **Harper-Arabie RM**, and Pavlik MC. 1999. Relating Reduction-Oxidation Chemistry of Wetlands to Variations in Hydrogeomorphic, Geochemical and Biological Structure, and Position in the Landscape. American Geophysical Union, San Francisco, CA.

Harper-Arabie RM and Kolm KE. 1998. An Integrated Approach to Wetlands Characterization in Determining Hydrologic Functions. The First International Symposium on Integrated Technical Approached to Site Characterization, Argonne National Laboratory, IL.

Glover KC, Kolm KE, **Harper-Arabie RM**, and Emerick JC. 1998. Determining hydrogeologic controls on a mountain wetland ecosystem using integrated ground water and water quality characterization, modeling, and sensitivity analysis. Geological Society of America Abstracts with Programs, vol. 30 no 7.

Harper-Arabie RM, and Kolm KE. 1998. A stepwise, Integrated Hydrogeomorphic Approach for the Classification of Wetlands and Assessment of Wetland Hydrological Function in the Southern Rocky Mountains of Colorado. Geological Society of America Annual Meeting, Toronto, Canada.

Harper-Arabie RM, and Kolm KE. 1997. The Pennsylvanian Mine Wetland: A Case Study Using an Integrated, Multidisciplinary Approach to Characterize Wetland Ecosystem Structure, Hydrology, and Biogeochemistry. Geological Society of America Annual Meeting, Salt Lake City, UT.

PROFESSIONAL AFFILIATIONS

Society of Environmental Toxicology and Chemistry, 1999 – present
Society of Wetland Scientists, 1998 – 2004
American Chemical Society, 2002 – present
Society of Women Engineers, 2000 – 2003
Sigma Xi Scientific Research Society, 2004 – 2012

PROFESSIONAL SERVICE

Book Proposal Reviews - *One or More Reviews for the Following Publishers*: College Publishing, Island Press, Elsevier, John Wiley and Sons.

Funding Reviews

EPA STAR Grants Reviewer, 2012
North Pacific Research Board (NPRB) Technical Peer Reviewer, 2012 and 2013
International Science and Technology Center *U.S. Civilian Research and Development Foundation* Reviewer, 2005
NSF Biogeosciences Program Proposal Reviewer, 2004
NSF Graduate Research Fellowship Program Reviewer, 2008, 2009, 2012 and 2013
NSF Population Dynamics Program Proposal Reviewer, 2007

Manuscript Reviews - *One or More Reviews for the Following Journals*: Archives of Environmental Contamination and Toxicology, Chemosphere, Environmental & Engineering Geoscience, Environmental Pollution, Environmental Toxicology and Chemistry, IEAM (Integrated Environmental Assessment and Management*)*, Georgia Journal of Science, Journal of Sea Research, Northwest Science, Risk Analysis, Science of the Total Environment, Soil and Sediment Contamination an International Journal.

Technical Reviews

Technical Reviewer for Teck Cominco Metals, Ltd. *Terrestrial risk modeling Level of Refinement 3*, 2006 – 2007
Technical Reviewer for Teck Cominco Metals, Ltd. *Aquatic Ecological Risk Assessment*, 2008

Boards and Advisory Committees

PNW-SETAC board, Academia At-Large representative, 2011 – present
Standard Methods Committee Appointment, 2008 – present
Capital Regional District Marine Monitoring Advisory Group (British Columbia), 2008 – 2010, 2013 - present
SETAC Science Sub-committee Member and Abstract Review Committee for North America National Meeting, 2010
Scientific Technical Services University Advisory Committee (WWU), 2005 – 2009

Meetings/Conferences

Discussion Leader on the C.R.E.A.T.E. method for teaching students how to read primary literature, Pacific Northwest Node of SCEWestNet Meeting, Seattle University 2013
Student Poster and Presentation Organizer, PNW-SETAC Annual Meeting 2013, 2014
Sessions Chair, Metals Toxicity and Puget Sound Issues, PNW-SETAC Annual Meeting, 2010
Student Poster and Presentation Judge, PNW-SETAC Annual Meeting, 2010

Workshop participant for West Coast Marine Research and Information Plan being developed by California, Oregon and Washington Sea Grant Program, 2007

Sessions Chair, Uranium Geochemistry Session, Uranium Geochemistry 2003 Conference, Nancy, France